GUSTATORY REJECTION THRESHOLDS FOR THE LARVAE OF THE CECROPIA MOTH, SAMIA CECROPIA (LINN.)

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Introduction

Dethier (1937, 1939) and Eger (1937) have so far conducted the only studies on gustation in lepidopterous larvae. Dethier, interested in localizing the gustatory receptors and in gustatory acuity and its relation to host selection by caterpillars, has determined acceptance thresholds for certain sugars and rejection thresholds for hydrochloric acid for a number of species. Eger, interested chiefly in determining the modalities of taste for caterpillars, has determined acceptance thresholds for some sugars and rejection thresholds for hydrochloric acid, sodium chloride, acetic acid, quinine hydrochloride, and saccharine. He concludes that there are probably only two modalities for caterpillars—acceptable and unacceptable, but he admits that his data offer no definite proof for this. The following experiments were undertaken to add more information to that already existing in this little known field.

Materials and Methods

The larvae of *Samia cecropia* used in the following experiments were reared from a single group of eggs laid by a gravid female captured in the early summer, the food plant being the sycamore (*Platanus occidentalis*). They were at first confined in a large jar and fresh leaves were added as needed, but later they were put on individual branches and numbered by marking with drops of colored paint. All the animals used in the experiments described below were in the last instar.

Rejection thresholds were determined, without disturbing the larvae, by placing drops of test solutions on the leaves as the animals fed. Since the caterpillars eat leaves in a very orderly fashion, it is easy to place drops so that they are reached by them within a very short time, usually about 10 to 15 seconds. Drops were placed on the leaves from small, new and carefully washed artists' brushes kept in the test solutions. A definite attempt was made to have the drops as nearly the same size as possible, but the importance of this is doubtful, for the larvae ordinarily ate on, undisturbed, below their thresholds and reacted almost immediately upon contact when the solutions were concentrated enough to bring about rejection.

The test solutions were prepared from C.P. chemicals, unless otherwise noted, using volumetric apparatus, and all tests were run within three hours after the solutions were prepared. The following chemicals were used, the concentrations prepared being given in parentheses after each: sucrose (2M, 1M, 0.5M), glucose, U.S.P. (2M, 1M, 0.5M), lactose, U.S.P. (1M, 0.5M), strychnine sulfate, U.S.P. (saturated solution), hydrochloric acid (0.4N, 0.2N, 0.1N, 0.075N, 0.050N, 0.025N), acetic acid (0.8N, 0.4N, 0.2N, 0.1N, 0.075N, 0.050N), sodium hydroxide

(0.4N, 0.2N, 0.1N, 0.075N, 0.050N), sodium chloride (2N, 1N, 0.75N, 0.50N, 0.25N), lithium chloride (4N, 2N, 1N, 0.75N, 0.50N), calcium chloride (1N, 0.75N, 0.50N, 0.25N, 0.10N), ammonium chloride (1N, 0.75N, 0.50N, 0.25N, 0.10N), and potassium chloride (1N, 0.75N, 0.50N, 0.25N, 0.10N).

There was no particular order in which these solutions were presented to the individual larvae. Since tests could be carried on only during feeding, it was found convenient to use three or four solutions simultaneously with all the caterpillars, testing the animals, whenever they fed, with the various concentrations. Thus there was no chance for associations to be built up by the caterpillars through consistent use of ascending or descending orders of concentrations. Each of the thresholds was determined at least three times for each of the caterpillars, and, in most cases, was determined five or six times, the greatest number for any individual being nine times. Acceptances are easy to determine, because the animals eat three or four "cuts" through the drop in their feeding. Rejections are usually equally sharp, because the caterpillars stop feeding immediately upon touching the drop, withdraw and start feeding elsewhere. It was possible, therefore, although only twelve specimens were used, to make accurate determinations for these solutions under these conditions.

The temperature of the laboratory was 23° C. and the relative humidity 70 per cent throughout all the work. Ordinary daylight entering the windows was the source of illumination.

RESULTS

There were no rejection thresholds for sugars or for strychnine sulfate, the larvae eating the most concentrated solutions of these offered to them. The thresholds for acids and salts used in these experiments are given in Table 1.

TABLE 1

Substance	Range	Mean	$\frac{1}{\text{Threshold}}$
HCl	0.04–0.15 N	0.083 ± .008 N	12
CH_3COOH	0.09–0.6 N	0.46 ± .05 N	
NaOH	0.09–0.15 N	0.13 ± .008 N	7.7
KCl	0.2 –0.9 N	0.45 ± .05 N	2.2
NH_4Cl	0.4 –0.6 N	0.46 ± .03 N	2.2
NaCl	0.4 –1.5 N	0.89 ± .1 N	1.1
$CaCl_2$	0.2 –0.9 N	0.61 ± .05 N	1.6
LiCl	0.9 –3.0 N	1.4 ± .15 N	0.72

In the column headed, "Range," are the lowest and highest thresholds discovered for the group; this does not imply a uniform distribution between these two extremes, but it gives some indication of the individual variations. In the column headed, "Mean," are the mean threshold normalities and the standard errors of these means (probable errors would be .6745 times the standard errors). In the column headed, "1/Threshold," are the reciprocals of the thresholds, these figures to be used below as indicative of stimulative efficiencies.

Thresholds were calculated as follows. The two critical concentrations—that is, the pair of concentrations, the lower of which was accepted, the higher rejected—were determined for each larva. Obviously, the true threshold lies between these two, and, for statistical purposes, it was assumed that the threshold lay midway between them. Thus, if a larva accepted 0.25N and rejected 0.50N, the threshold assigned was 0.38N, if between 0.50N and 0.75N, the threshold assigned was 0.63N. Since the second digit cannot be interpreted literally in such a system, these thresholds are designated in the table as 0.4N and 0.6N respectively, only the means being given to the second place.

While the validity of using the midpoint betweeen the two critical concentrations as the threshold for an individual animal can be questioned, certain supplementary observations and considerations made it appear permissible to use this for the calculation of means. Thus, most of the animals readily ate through the drop of lower concentration and stopped clearly on reaching the drop of higher concentration, indicating a threshold in the middle range between the two. A few, however, hesitated at the lower concentration before continuing with feeding and reacted violently at the higher, drawing back sharply and often ceasing feeding for a time, indicating a threshold near the lower concentration. Others ate readily through the lower concentration and only hesitated at first at the higher, made a few tries at eating it, but finally stopped and usually simply moved a short distance away on the same leaf, indicating a threshold near the higher concentration. Since these latter two cases seemed to be of equal occurrence, with the majority of rejections of the first type described, it seemed fair, for purposes of calculation of means for the group to be used for comparative purposes only, to assign the midpoints between the critical concentrations as the thresholds for the individuals, in the group, without implying that these represent true thresholds for any of the individuals.

DISCUSSION

Sugars and strychnine sulfate

Since there were no rejection thresholds for sugars or for strychnine sulfate, no conclusions can be drawn concerning the ability of the caterpillars to taste these substances. It must be recognized that failure of rejection does not imply that the animals cannot taste these compounds, just as rejection thresholds do not measure the lowest concentrations that can be tasted. To me, sycamore leaves tasted almost as bitter as the strychnine solution, and, since these larvae feed on many species of trees, it would seem logical that their rejection threshold for bitter substances would be high. Dethier (1937) has shown definitely that caterpillars of this species can taste glucose and sucrose, but he used acceptance thresholds in his work.

Acids

The acid thresholds here given are higher than those given by Dethier and Eger, but that is to be expected, for the acids in their experiments were offered in drops of water, while in this case there was some mixing of the acids with the food. The pH of the solution of hydrochloric acid corresponding to the mean rejection threshold normality is 1.2, while the pH of the solution of acetic acid at the mean rejection threshold normality is 2.5. Thus, acetic acid, as for man, is much more effective a stimulating agent than is hydrochloric acid at the same pH. The ratio for this species is 20:1, which compares favorably with Eger's (1937) report for another species of a ratio averaging about 23:1. This ratio is comparable to that for humans (28:1), and Eger suggests that it indicates a similarity in the buffering capacities of the salivas of the two species. It further, however, suggests a similarity of action in both, and may indicate a sensitivity to hydronium ion in these caterpillars corresponding to the sour taste in humans.

Salts

The order of relative stimulative efficiencies, as measured by the reciprocals of the normalities of the rejection limens, for the various cations, as chlorides, gives the series: $NH_4^+ = K^+ > Ca^{++} > Na^+ > Li^+$.

This series can be verified further by checking the orders for the twelve individuals to determine in how many cases any specific cation has an equal, greater, or lesser stimulative efficiency than any other cation. Using this method, it is found that K^+ has a greater efficiency than NH_4^+ ($K^+ > NH_4^+$) in 4 individuals, NH_4^+ has greater than K^+ ($K^+ < NH_4^+$) in 3 individuals, and K^+ is equal to NH_4^+ ($K^+ = NH_4^+$) in 5 individuals. This indicates that K^+ and NH_4^+ are equal in stimulative efficiency. Comparing K^+ and NH_4^+ with Na^+, the following orders are noted: $K^+ > Na^+ - 9$, $K^+ = Na^+ - 3$, $K^+ < Na^+ - 0$, and $NH_4^+ > Na^+ - 8$, $NH_4^+ = Na^+ - 4$, $NH_4^+ < Na^+ - 0$. These clearly show that K^+ and NH_4^+ have greater stimulative efficiencies than Na^+, and further confirm their equality. Comparing Na^+ with Li^+, the following are found: $Na^+ > Li^+ - 9$, $Na^+ = Li^+ - 3$, $Na^+ < Li^+ - 0$. Obviously, Na^+ is more stimulating than Li^+. K^+ and NH_4^+ are

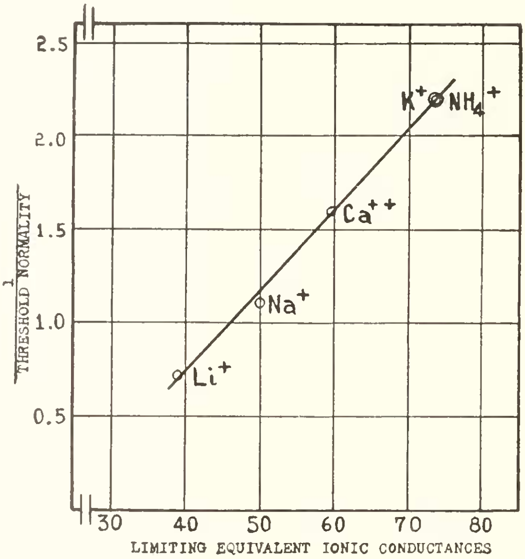

FIGURE 1. Showing the relationship between the stimulative efficiencies of the cations, as chlorides, and their limiting equivalent ionic conductances in Mhos at 25° C. Values for the latter are from Gucker and Meldrum (1942).

found to be more stimulating than Li^+ in all cases, thus verifying their position above Na^+. Ca^{++} shows the greatest variation in position. Comparing with NH_4^+ or K^+, the following are found: K^+ or $NH_4^+ > Ca^{++} - 8$, K^+ or $NH_4^+ = Ca^{++} - 2$, K^+ or $NH_4^+ < Ca^{++} - 2$. Thus K^+ and NH_4^+ have greater stimulative effects than Ca^{++}. Comparing Ca^{++} with Na^+ we find: $Ca^{++} > Na^+ - 9$, $Ca^{++} = Na^+ - 0$, $Ca^{++} < Na^+ - 3$. Ca^{++} is thus more stimulating than Na^+. With Li^+ we find: $Ca^{++} > Li^+ - 11$, $Ca^{++} = Li^+ - 1$, $Ca^{++} < Li^+ - 0$; Ca^{++} is thus more stimulating than Li^+. All of these individual orders considered together indicate the same arrangement as that found using the mean thresholds, namely: $NH_4^+ = K^+ > Ca^{++} > Na^+ > Li^+$.

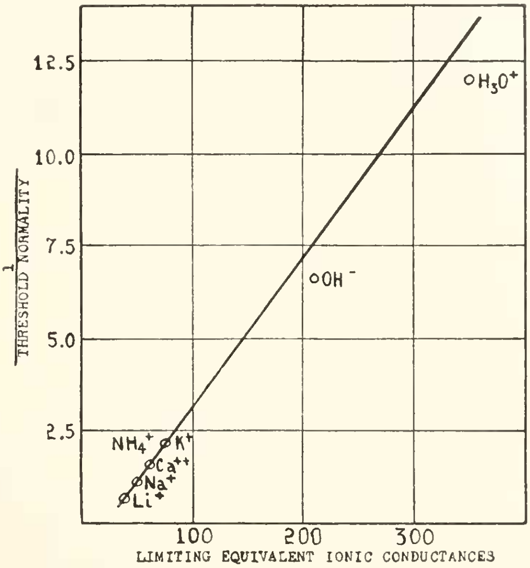

FIGURE 2. Showing the relationship between the stimulative efficiencies and the limiting equivalent ionic conductances in Mhos at 25° C. of certain ions. The slope is taken as determined by the metallic ions. Values for the conductances are from Gucker and Meldrum (1942).

Hopkins (1932) has tabulated the discoveries of numerous investigators concerning stimulative efficiencies of various cations and anions. The order shown in the case of the caterpillars of *Samia cecropia* here used is similar to that discovered for other species of animals. This suggests some basic chemical or physical reason for this order. Hopkins relates the order of cations for the oyster, which he studied, to the atomic weights and atomic mobilities. In the present case, there is no obvious relationship between ionic weights and stimulative efficiences, but Figure 1 shows the close agreement between the stimulative efficiencies of the cations, as determined by the reciprocals of the normalities of the rejection limens, and their limiting equivalent ionic conductances which are directly proportional to the ionic mobilities, in fact, have sometimes been called mobilities. Since the chloride ion is the anion in all cases, it is impossible to determine its relative stimulative effect, and it is, therefore, disregarded in this plot, since, whatever its efficiency, it is constant for all substances tested.

These relationships suggest a common mode of action, and possibly a common modality of taste, for all the cations tested. Since, however, this same order is found for man for K^+, Na^+, and Li^+, and KCl has a taste easily distinguishable from NaCl or LiCl, this conclusion is not warranted without further evidence.

Sodium hydroxide shows a much greater stimulative efficiency than any of the other salts[1] used in this work, thus indicating that the OH^- ion is the critical one in its effectiveness, since some measure of the efficiency of the Na^+ ion is given by the stimulative efficiency of NaCl. Comparison of the data for NaOH and HCl shows that the OH^- ion is much less stimulating than the H_3O^+ ion.

Figure 2 shows the extrapolation of the graph, stimulative efficiency vs. limiting equivalent ionic conductance to include OH^- and H_3O^+. The value for the stimulative efficiency of the OH^- ion is approximated by subtracting from the stimulative efficiency for NaOH the stimulative efficiency for NaCl, assuming thus that most of this is due to the Na^+ ion. This shows that there is a possibility that OH^- and H_3O^+ fall in the same series. That they are apparently lower in stimulative efficiency than is needed for a perfect "fit" in the graph would be expected from the fact that the solutions containing them were mixed with the protoplasm of the leaves and with the saliva of the caterpillars, both of which are buffered. This might mean that there is only an "unacceptable" modality of taste for all these substances, as Eger (1937) suggests, but this cannot be decided without further evidence. The OH^- and H_3O^+ ions, in the case of man, show a high stimulative efficiency when compared with other anions and cations, and stimulation by the H_3O^+ ion, at least, is admitted to be the important factor in the sour taste, as opposed to the salt taste of the metallic chlorides.

In all, these results can only be taken as suggestive and indicative of a need for much more extensive and careful work on the gustatory responses of animals to more than the usual NaCl in testing the salt taste.

SUMMARY

Rejection thresholds for HCl, CH_3COOH, NaOH, NaCl, NH_4Cl, KCl, $CaCl_2$, and LiCl, presented as drops of solutions on leaves of the food plant, were determined for caterpillars of the cecropia moth, *Samia cecropia*. Rejection thresholds for glucose, sucrose, lactose, and strychnine sulfate either do not exist under these conditions, or are higher than the saturation concentrations of solutions of these substances. The lowest threshold of those tested is that for HCl, but CH_3COOH has greater stimulative efficiency when it is compared with HCl at the same pH. The threshold for NaOH is higher than that for HCl, indicating that the OH^- ion is less stimulating than the H_3O^+ ion. The order of stimulative efficiency for the cations, as chlorides, is $NH_4^+ = K^+ > Ca^{++} > Na^+ > Li^+$. This is the order of ionic mobilities to which the stimulative efficiencies seem to be related. No conclusions can be drawn with certainty as yet regarding the modalities of taste for these animals.

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[1] Modern Acid-Base concepts require that NaOH be considered as a salt.

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